

WHAT IS CLAIMED IS:

Sub B 1. A method of providing a composition comprising a mixture of cells derived from human liver tissue, which mixture comprises an enriched population of human liver progenitors, the method comprising:

- providing a substantially single cell suspension of human liver tissue comprising a mixture of cells of varying sizes, including immature cells and mature cells; and
- debulking the suspension under conditions that permit the removal of mature cells and those of relatively large size, while retaining immature cells and those of relatively small size,

to provide a mixture of cells comprised of an enriched population of human liver progenitors which human liver progenitors themselves, their progeny, or more mature forms thereof, exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

2. The method of claim 1, in which the liver tissue is obtained from a fetus, a neonate, an infant, a child, a juvenile, or an adult.

3. The method of claim 1 in which the immature cells have a diameter less than about 15 microns.

4. The method of claim 1 in which the enriched population comprises human diploid liver cells.

5. The method of claim 1 in which the liver progenitors are hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or mixtures thereof.

6. The method of claim 1 in which the alpha-fetoprotein is full-length alpha-fetoprotein.

7. The method of claim 1, in which the debulking comprises separation according to cell size, buoyant density, or a combination thereof.

8. The method of claim 1 in which the debulking step comprises centrifugal elutriation, density gradient centrifugation, panning, affinity

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chromatography, tagging with fluorescent labels, countercurrent fluid flow, continuous-flow centrifugation, zonal centrifugation, use of magnetic beads, or combinations thereof.

9. The method of claim 1 which further comprises selective lysis of
5 the mature cells.

10. The method of claim 1 which further comprises selecting those
cells, which themselves, their progeny, or more mature forms thereof exhibit one
or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

Sub C3 → 11. A human liver progenitor isolated by the method of claim 1.

Sub C3 → 12. A method of providing a composition comprising an enriched
population of human liver progenitors comprising:

(a) providing a substantially single cell suspension of human liver tissue,
and

(b) subjecting the suspension to a positive or negative immunoselection.

15 13. The method of claim 12 in which the liver progenitors are hepatic
progenitors, hemopoietic progenitors, mesenchymal progenitors, or combinations
thereof.

20 14. The method of claim 12 in which the immunoselection comprises
selecting cells that express markers associated with hemopoietic cells, cells that
express markers associated with hepatic cells, cells that express markers
associated with mesenchymal cells, or combinations thereof.

25 15. The method of claim 12 in which the immunoselection comprises
selecting from the suspension those cells, which themselves, their progeny, or
more mature forms thereof exhibit one or more markers indicative of expression
of alpha-fetoprotein, albumin, or both.

16. The method of claim 15 which further comprises selecting those
cells which themselves, their progeny, or more mature forms thereof produce full-
length alpha-fetoprotein mRNA.

30 17. The method of claim 12 in which the immunoselection comprises
selecting from the suspension those cells that express an adult liver cell-specific

marker.

18. The method of claim 12 in which the immunoselection comprises selecting those cells, which themselves, their progeny, or more mature forms thereof express CD14, CD34, CD38, ICAM, CD45, CD117, glycophorin A, 5 connexin 32, osteopontin, bone sialoprotein, collagen I, collagen II, collagen III, collagen IV, or combinations thereof.

19. The method of claim 12 which the immunoselection comprises selecting those cells, which themselves, their progeny, or more mature forms thereof further express alpha-fetoprotein-like immunoreactivity, albumin-like 10 immunoreactivity, or a combination thereof.

Sub C 4 20. A human liver progenitor isolated by the method of claim 14.

Sub B 4 21. A composition comprising an enriched population of human liver progenitors, their progeny, or more mature forms thereof, which human liver exhibit one or more markers indicative of expression of alpha-fetoprotein, 15 albumin, or both.

22. The composition of claim 21 in which the progenitors comprise hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or combinations thereof.

23. The composition of claim 21 in which the progenitors, their 20 progeny, or more mature forms thereof express CD14, CD34, CD38, CD117, ICAM or combinations thereof.

24. The composition of claim 21 in which the progenitors harbor exogenous nucleic acid.

25. The composition of claim 24 in which the exogenous nucleic acid 25 encodes at least one polypeptide of interest.

26. The composition of claim 24 in which the exogenous nucleic acid promotes the expression of at least one polypeptide of interest.

27. A method of treating liver dysfunction or disease responsive to treatment with liver progenitors in a subject in need thereof, comprising 30 administering to the subject an effective amount of human liver progenitors, their

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progeny, more mature forms thereof, or combinations thereof, in a pharmaceutically acceptable carrier and treating the liver dysfunction or disease.

28. The method of claim 27 in which the human liver progenitors comprises hepatic progenitors, hemopoietic progenitors, mesenchymal progenitors, or combinations thereof.

29. The method of claim 27 further comprising administering simultaneously or sequentially in any order an effective amount of adult human liver progenitors, their progeny, more mature forms thereof, or combinations thereof.

10 30. The method of claim 27 in which the human liver progenitors are administered parenterally.

31. The method of claim 27 in which the liver disorders or dysfunctions comprise hepatocholangitis, hepatomalacia, hepatomegalias, cirrhosis, fibrosis, hepatitis, acute liver failure, chronic liver failure, cancer, 15 hematologic disorders, hematologic dysfunctions, or inborn errors of metabolism.

32. The method of claim 31 in which the cancer comprises hepatocarcinoma, hepatoblastoma, or both.

33. The method of claim 31 in which the cancer comprises a metastatic tumor in liver deriving from a primary site selected from the group consisting of 20 intestine, prostate, breast, kidney, pancreas, skin, brain, and lung.

34. The method of claim 31 in which the hematologic disorders or dysfunctions include anemia, leukemia, or those induced by chemotherapy, radiation, drugs, viruses, trauma, or combinations thereof.

35. A method of treating a disease in a subject in need thereof 25 comprising administering an effective amount of human hepatic progenitors, their progeny, or more mature forms thereof in which the human hepatic progenitors, their progeny, or more mature forms harbor exogenous nucleic acid.

36. A bioreactor comprising the composition of claim 21 and at least one compartment having culture medium.

30 37. The bioreactor of claim 37 in which the bioreactor is adapted for

use as an ~~artificial~~ liver.

38. A cell culture comprising the composition of claim 21, an extracellular matrix component, and a culture medium.

39. A pharmaceutical composition comprising the composition of claim 21 and a pharmaceutically acceptable carrier.

40. A method for cryopreservation of adherent cells comprising:

- providing adherent cells in an extracellular matrix or in a culture medium comprising a viscosity enhancer;
- suspending the cells in a cryopreservation mixture comprising culture medium, an ice-crystal inhibitor, a carbohydrate regulating factor, an iron donator, a lipoprotein, and a lipid; and
- cooling the suspension to below the freezing point of the cells.

41. A cryopreservative mixture for preservation of adherent cells comprising culture medium, an ice-crystal inhibitor, a carbohydrate regulating factor, an iron donator, a lipoprotein, and a lipid.

Sub B 42. Human liver progenitors, their progeny or more mature forms thereof which exhibit one or more markers indicative of expression of alpha-fetoprotein, albumin, or both.

43. Human liver progenitors, their progeny or more mature forms thereof which exhibit the phenotype glycophorin A⁺, CD45⁺, alpha-fetoprotein⁺⁺⁺, albumin⁺, and ICAM⁺.

44. The human liver progenitors of claim 43 which further express CD14⁺, CD34⁺⁺, CD38⁺⁺, CD117⁺, or combinations thereof.

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